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Development of biohydrogen production by photobiological, fermentation and electrochemical processes: A review

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ABSTRACT

Production of biohydrogen has the potential to be a renewable alternative to current technologies. There are varieties of technologies for biological hydrogen production mechanisms including biophotolysis, photo fermentation, dark fermentation and hybrid biohydrogen production by electrochemical processes. In these studies, a review on the recent developments of biohydrogen production is presented. First, the theoretical principles of biophotolysis by cyanobacteria and green micro algae, as well as direct and indirect of biophotolysis process on hydrogen production are described. Secondly, practical aspects and fundamental of biological hydrogen production processes by photo and dark fermentation are reviewed. This work also involved comparison of the maximum H_2 yield, bacterial strains, operating condition, suitable substrates, and mathematical models for fermentative hydrogen production. A new hybrid biological hydrogen production processes by using the electrochemical process is then proposed. This study can also be used to improve the basic and current knowledge about the performance of the biophotolysis, fermentative and electrochemical process in producing hydrogen gas as the alternate fuel.

Contents

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1. Introduction

One of the great challenges in the coming decade is how to get new renewable energy sources that are environmentally friendly and to replace high dependency on fossil fuels. Until recently, almost all of the energy needed is derived from the conversion of fossil energy sources, such as for power generation, industrial and transportation equipment that uses fossil fuels as a source of energy. Fossil fuels are source of non-renewable energy and also have seriously negative impacts on the environment, e.g. soil, water, air, and climate. The use of fossil fuels cause excessive global climate change because emissions of greenhouse pollutants and the formation of compounds CO_x, NO_x, SO_x, C_xH_y, ash, and other organic compounds that are released into the atmosphere as a result of combustion [1,2].

Based on the above considerations, in recent years various studies has been conducted to obtain a sustainable source of energy that can replace fossil fuels and which do not have a negative impact on the environment. Hydrogen is one alternative fuel substitute for fossil fuels and is considered as an "energy carrier" with a promising future. It has a high energy content of 122 kJ/g, which is 2.75 times greater than hydrocarbon fuels [3].

Hydrogen plays a very important role and contribution in the global era that is based on clean renewable energy supplies and sustainably which will provide major contributions to the world economic growth. Hydrogen fuel is environmentally friendly, clean and is the most abundant element in the universe in its ionic form. Hydrogen gas is also colorless, tasteless, odorless, light and non-toxic. When its gas is used as fuel, it will not produce pollution to the air but it produces only water as its end-product when it burns [4]. Hydrogen gas which is produced by biological processes becomes very interesting and promising because they can be operated at ambient temperature and pressure with minimal energy consumption, and become more environmentally friendly [5].

According to Mohan et al. [5], hydrogen can be produced from different types of raw materials, including fossil fuels, water, and biomass. Hydrogen production from renewable sources can be obtained in different ways. There are several major renewable energy sources to produce from the water that flows, the heat from the earth, wind, solar, biomass and biological hydrogen production from microorganisms. Many microorganisms are known to produce hydrogen under certain conditions, including microalgae such as blue-green algae that use light energy to split water for hydrogen formation and cyanobacteria that usually use carbohydrates to store energy from photosynthesis to produce hydrogen from water [6].

Production of hydrogen gas from renewable biomass materials can be obtained from a variety of organic-based starch industry waste, industrial waste biodiesel, lignocellulosic materials such as wood and its products, food, household waste and others. Biological hydrogen production using carbohydrate-rich biomass as a renewable resource is one of the alternative methods where processes can take place through an anaerobic process (dark fermentation) and photosynthesis process (photo-fermentation). Dark fermentation is the conversion of organic compounds to hydrogen; it takes place in the absence of oxygen by a group of bacteria using multi enzyme systems. This process takes place in several stages, where a series of complex biochemical reactions manifested by a group of bacteria into hydrogen gas. The first step is the enzymatic hydrolysis of high molecular weight organics to water-soluble organics, and in a second step the simple organic to produce Volatile Fatty Acids (VFA), hydrogen, and carbon dioxide [7,8].

Photo-fermentation is the conversion of organic compounds to biohydrogen involving various groups of bacteria photosynthetic by a series of biochemical reactions. Photo-fermentation differs from dark-fermentation because it only occurs in the presence of light. If viewed from the perspective of economic, hydrogen production through dark-fermentation has advantages and more profitable than photo-fermentation processes because of its ability to continuously produce hydrogen and does not depend on energy provided by sunlight [9].

A new hybrid biological hydrogen production processes has been developed very recently by use of the electrochemical process. These processes include the electrolysis which is based on the concept and practice of Microbial Fuel Cell (MFC). This method needs to be added with electric potential generated by a microbial fuel cell, so as to achieve sufficient strength to release protons to hydrogen. Production of hydrogen by an electrochemical process is not limited only to carbohydrates, as in the fermentation process. Other biodegradable organic matter dissolved can be used to generate hydrogen from the complete oxidation of organic matter. Instead, by electrochemically increasing the cathode potential in a Microbial Fuel Cell (MFC), it is possible to continuously produce hydrogen assisted electron exchange by bacteria. This method greatly decrease the amount of energy needed to produce hydrogen from organic matter compared to hydrogen production from water via electrolysis [10,11].

This review focuses on literature survey carried out on the production of hydrogen by biological process. This literature study will discuss in detail about the biological hydrogen production methods including biophotolysis, photo-fermentation and dark-fermentation and hybrid biological hydrogen production by electrochemical processes.

2. Fundamentals of biological hydrogen production processes by biophotolysis

Biophotolysis is associated with plant-type photosynthesis process, formerly known as blue-green algae that uses light to split water for hydrogen formation, and takes place under anaerobic conditions. Biophotolysis indirectly involve cyanobacteria usually use carbohydrates to store energy from photosynthesis to produce hydrogen from water.

2.1. Biophotolysis of water by cyanobacteria and green micro algae

Biophotolysis process can occur in various species of bacteria and algae, for example species of bacteria and algae that can produce hydrogen through biophotolysis like photosynthetic bacteria from soil or natural water, Anabaena species Cyan bacteria, or eukaryotic alga Chlamydomonas species Reinhardt. The hydrogen gas production in a sustainable and environmentally friendly to produce clean energy from renewable resources can be obtained through biophotolysis of water by cyanobacteria and Green Micro Algae. Cyanobacteria and green algae can split water into hydrogen and oxygen molecules by using sunlight [12,13]. Mechanism of biohydrogen production through biophotolysis or photoautotrophic process is hydrogen gas formed from the water by using sunlight and CO_2 as the sole source for energy through the process of hydrogenase enzyme by bacteria and algae [14]. Fig. 1 shows the ability to photosynthesis produce H_2 under anaerobic conditions using green alga *Chlamydomonas reinhardtii*.

The advantage of biophotolysis is that, there is no requirement of adding substrate as nutrients. Water is the primary electron donor required for the production of hydrogen gas. Sunlight and CO_2 are the basic inputs needed to grow the cyanobacteria or microalga on biophotolysis process through the hydrogenase enzyme.

Production of hydrogen gas by green algae and cyanobacteria is one of the methods that produce renewable energy which does not emit greenhouse gas effect with the availability of abundant resources, namely water as substrate and solar energy as a source of energy. Thus, hydrogen gas produced could be used in a fuel cell to generate electricity as shown in Fig. 2 [15].

In the biophotolysis process, light energy is absorbed by photosystem (PSI and PSII) of microalgae; this energy is then transferred through the electron transport chain, in turn reducing ferredoxin and provides electrons to the hydrogenase enzyme. In certain circumstances such as in anaerobic conditions, for example at a pressure of hydrogen is very low or low light, hydrogenases can provide a solution for excess electrons when carbon fixation component of the photosynthetic chain is disrupted [16].

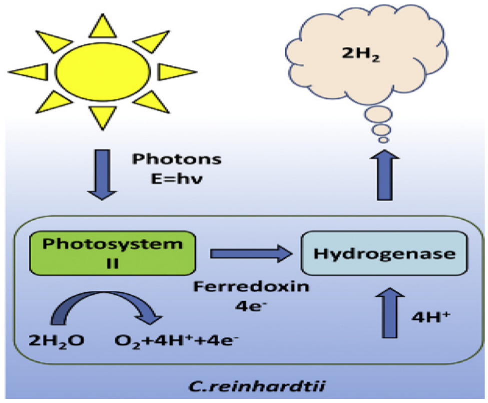


Fig. 1. The green alga *Chlamydomonas reinhardtii* has the ability to photosynthetically produce H_2 under anaerobic conditions.
Excerpted from Tamburic et al. [22].

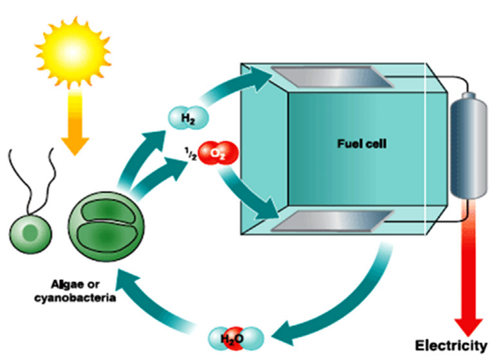


Fig. 2. Schematic representation of the vision for photobiological H_2 production and its utilization in a H_2 fuel cell.
Adapted from Maness et al. [15].

Many studies have been reported for the hydrogen production via biophotolysis, for example in Table 1 shows the various conditions of optimum and maximum production rate of hydrogen production by green micro algae and cyanobacteria. The study of hydrogen evolution as a consumption of reducing equivalent in green algae *Chlamydomonas* MGA 161 was introduced by Ohta et al. [17]. Demonstration of sustained hydrogen photoproduction by Immobilized, Sulfur-Deprived *C. reinhardtii* using CO_2 and acetate as carbon source with light intensity of 100 ($\mu E/m^2/s$) is also observed by Laurinavichene et al. [18]. There are several types of bacterial strains in green algae that can be used in biophotolysis process such as *Platymonas subcordiformis* [19], *Chlamydomonas reinhardtii 137c* [20], *Chlorella sorokiniana Ce* [21], and *Chlamydomonas reinhardtii* CC-124 [22].

The hydrogen metabolism of mutated forms using cyanobacteria *Anabaena variabilis* ATCC 29413 in continuous cultures and under nutritional stress was introduced by Sveshnikov et al. [23] using N_2 (25%) and CO_2 (2%) as carbon source and light intensity of 140 ($\mu E/m^2/s$). Berberoglu et al. [24] has investigated the effect of nutrient media on photobiological hydrogen production by *Anabaena variabilis* ATCC 29413 using H_2O (95%) and CO_2 (5%) as carbon source and light intensity of 150 ($\mu E/m^2/s$). Hydrogen production from different microbes in cyanobacteria such as *Anabaena azollae* [25], *Chroococcidiopsis thermalis* CALU 758 [26], *Anabaena* PCC 7120 and AMC 414 [27], *Synechococcus sp.* Strain H-1 [28] and *Arthrospira sp. PCC 8005* [29] has also been reported in Table 1.

A hydrogenase is an enzyme that catalyses the reversible oxidation of molecular hydrogen. The main purpose of studying about the hydrogenase is to understand the mechanism of hydrogen production, control of cell metabolism, and ultimately increase the production of hydrogen. Hydrogenases play a vital role in biophotolysis by Cyanobacteria and Green Micro Algae [30,31]. Hydrogenases were classified according to metals thought to be at their active sites; three classes were recognized: iron-only ([FeFe]-hydrogenases), nickel-iron ([NiFe]-hydrogenases), and metal-free hydrogenses [32]. Among the three types of enzymes most commonly found in various bacterial and algae are [FeFe]-hydrogenases and [NiFe]-hydrogenases except for metal-free hydrogenases found in some types of methanogens. Three types of this enzyme are monomeric [FeFe]-hydrogenases most involved in the evolution of hydrogen, features high sensitivity to oxygen (O_2) and carbon monoxide (CO) [31,33].

2.1.1. FeFe-hydrogenases

[FeFe]-hydrogenase is an enzyme which plays a vital role in anaerobic metabolism, which is produced by green algae and

Table 1
Comparison of the optimum condition and maximum production rates of hydrogen production by cyanobacteria and green micro algae (laboratory photobioreactor).

Organism	Bacterial strains	Carbon source/gas for growth	Light intensity ($\mu E/m^2/s$)	Optimum condition		H_2 production rate (ml L_{cult}^{-1} h^{-1})	Refs.
				pH	T (°C)		
Green microalgae	*Chlamydomonas MGA 161*	CO_2: 5%, water: 95%	115	8	30	4.48	[17]
	Platymonas subcordiformis	Air; seawater nutrients	101	8	25	0.05	[19]
	Chlamydomonas reinhardtii CC-124	CO_2: 3%, water: 97%, acetate: 17 mM	100	7	28–30	2.2	[18]
	Chlamydomonas reinhardtii 137c	Acetate-phosphate	110	7.2	25	2.5	[20]
	Chlorella sorokiniana Ce	Acetate	120	7.2	30	1.35	[21]
	Chlamydomonas reinhardtii CC-124	Water CO_2	< 200	4–9	20	1.1	[22]
Cyanobacteria	*Anabaena variabilis ATCC 29413*	CO_2: 2%, N_2: 25% Ag: 73%	140	7.5	30	13	[23]
	Anabaena azollae	CO_2: 2%	140	-	-	13	[25]
	Chroococcidiopsis thermalis CALU758	CO_2: 1%, water: 99%	70	7.5	26	4.03	[26]
	Anabaena PCC 7120 and *AMC 414*	CO_2: 2%, water: 98%	110–220	8	30	14.9	[27]
	Anabaena variabilis ATCC 29413	CO_2: 5%, water: 95%	150	6.9–7.5	30	0.9	[24]
	Synechococcus sp. Strain H-1	CO_2: 6%, water: 94%	100	8–8.5	55	0.9	[28]
	Arthrospira sp. PCC 8005.	Fe^{2+}: β-mercaptoethanol	40	7	30	5.91	[29]

become more efficient catalyst hydrogenases. [FeFe]-hydrogenase is able to catalyses the reversible oxidation of molecular hydrogen. From Fig. 3A, we can see that the FeFe-hydrogenases only contains a dinuclear iron center that is attached to a protein with only one bond between cysteine residues and one of the two iron atoms. [Fe–Fe]-hydrogenases contain [2Fe-2S] and additional [4Fe-4S] cluster, an electron shuttle between sites the hydrogen activate, in proteins, and redox partners on the surface. Cysteine also functions as a ligand to a cluster of adjacent [4Fe-4S], so there is a sulfur bridge between two metal sites [15]. Iron atoms from binding [4Fe-4S] center to the structure of proteins by three additional cysteine residues and linked through a protein cysteine residue to a 2Fe subcluster. Except for cysteine bridging cysteine, the iron atoms of the 2Fe center coordinated to carbon monoxide (CO) and cyanide (CN) ligands. With the CO and CN is expected to allow for stabilization of low oxidation and spin state of iron is required for activity [34].

2.1.2. Cyanobacterial NiFe-bidirectional hydrogenases

[NiFe]-hydrogenases produced by cyanobacteria consist of the center of several metals, including Ni–Fe bimetallic sites active, iron-sulfur and Mg^{2+} ions. Ni–Fe active site is located inside the protein molecules and functions as bidirectional hydrogenases that involve a number of lines in the catalytic reaction route like: route of electron transfer, proton transfer lines and gas-access channels [35–38].

[NiFe] hydrogenases function as the metabolism of hydrogen, which are grouped into two sub units that are; hydrogenase large and small. Large subunit contains a core double [NiFe] active site and the small subunit binds at least one [4Fe-4S] cluster [39]. While the large subunit [NiFe]-hydrogenase and other nickel metallo-enzymes synthesized as a precursor without metal active sites that experienced a post-translational maturation process of the complex [40,41]. Synthesis and insertion of metallocentre of NiFe-hydrogenases is a complex process, involving at least seven proteins and chemical components such as Adenosine triphosphate (ATP), Guanosine triphosphate (GTP), and karbamoilfosfat, which is the embryo of cyanide [40].

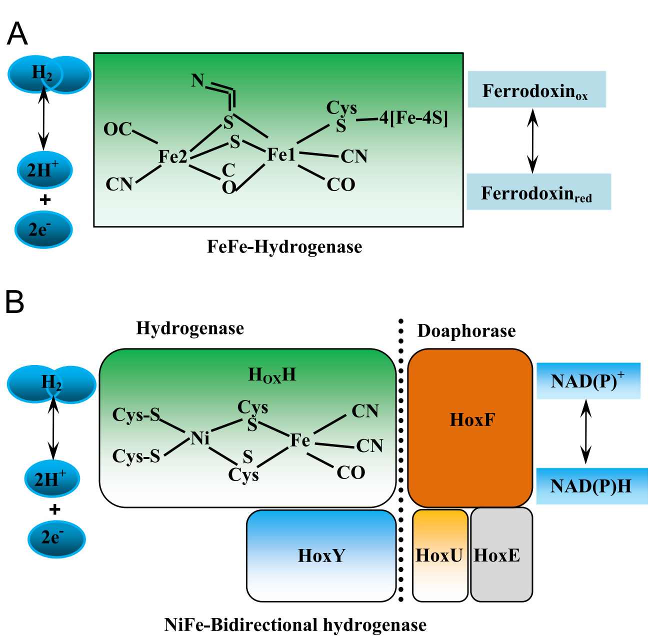


Fig. 3. Schematic representation of the [FeFe]-hydrogenases and [NiFe]-hydrogenases.
Adapted from Maness et al. [15].

The NiFe-hydrogenases have higher levels of similarity and the complex among all the hydrogenase operons and FeFe-hydrogenases, so that the microbes have a very important role in hydrogen production process. In Fig. 3B, we can see that the bidirectional NiFe-hydrogenase of cyanobacteria that consists of five subunits. Large as the center of the catalytic subunit of pentameric hydrogenase HoxH and containing atoms of Fe and Ni associated with the ligands CN and CO and sulfur atoms. While the small subunit hydrogenase, HoxY, contains a cluster [4Fe-4S] that are required to transfer electrons to the large catalytic subunit. For the remaining three subunits that form part of the complex is HoxF diaphorase, HoxU, and HoxE and function as an electron channel between the NAD (P) H and hydrogenase active site. The large number of genes involved in the maturation of the structural subunit of NiFe-hydrogenases, an indication of the complexity of the molecular structure of hydrogenase [15].

2.2. Direct biophotolysis

Direct biophotolysis is a biological process that can produce hydrogen directly from water using microalgae photosynthesis system to convert solar energy into chemical energy in the form of hydrogen, the reaction is generally as follows:

$$2H_2O + \text{Solar energy} \overset{(\text{Photosynthesis})}{\rightarrow} 2H_2 + O_2 \quad (1)$$

In indirect biophotolysis green algae or cyanobacterium (Fig. 4), hydrogen gas is produced through photosynthesis by using solar energy to split water molecules. In this process also decrease ferredoxin, hydrogenase or nitrogenase which these compounds are very sensitive to oxygen [42].

The advantage of this process is that, even in low light intentitas, green algae and anaerobic conditions are still able to convert almost 22% of light energy by using the hydrogen as an electron donor in the process of fixation of CO_2. From the results of further studies, even photosystem I-defective mutants of Chlamydomonas are able to produce efficiency twice as large as the wild type strain. Hydrogen production by green microalgae take place in anaerobic conditions in the dark to induce activation of enzymes involved in hydrogen metabolism. Hydrogenase sensitivity to oxygen is a big challenge for this method, so that further research is needed to develop engineered hydrogenase so that it is not sensitive to oxygen inactivation. Green microalgae have the genetic machinery, enzymatic, metabolic, and electron-transport to photoproduce hydrogen so that hydrogenase is able to combine a proton (H^+) in media with and release electrons to form hydrogen.

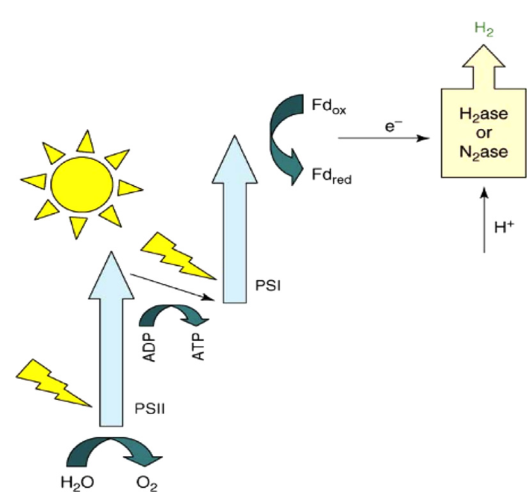


Fig. 4. Direct biophotolysis of green algae or cyanobacteria.
Adapted from Hallenbeck [42].

Synthesis hydrogen permits the flow of electrons through the electron transport chain, which supports the synthesis of Adenosine Triphosphate (ATP) [14].

In photosynthesis, photosystem process occurs in two-stage process, photosystem I (PS I) and photosystem II (PS II), both processes operate in series. In anaerobic conditions (lack of oxygen), electron (e^-) from reduced ferredoxin (Fd) is used by the hydrogenase to reduce protons (H^+) and evolve hydrogen. Partial inhibition of PS II can produce anaerobic conditions for the cells in the photobioreactor, because there are less water oxidation activities to evolve O_2 and residues used by respiration [43].

$$2H_2O + \text{light energy} \rightarrow O_2\uparrow + 4H^+ + Fd(red)(4e^-) \rightarrow Fd(red)(4e^-) + 4H^+ \rightarrow Fd(ox) + H_2 \quad (2)$$

In photosystem I (PS I) generate reductant for CO_2 reduction while in photosystem II (PS II), the separation of water and oxygen. In PS II, P680, the strongest absorption by the antenna pigments at wavelengths less than 680 nm (due to photon excitation energy of each) and then transferred to the PS II reaction center and produces a strong oxidant that is able to liberate electrons from water. Reductant that provides reducing equivalents through a series of electron carriers and cytochrome complex to the oxidized reaction center of PS I. While the PS I reaction center, the strongest absorption by antenna pigment at wavelength 700 nm, P700. The light energy absorbed by PS I is not only used to oxidize the reaction center, but also to produce a strong reductant capable of reducing oxidized nicotinamide adenine dinucleotide phosphate (NADP+) to NADPH [44].

Fig. 5 illustrates the mechanism of sulfur deprivation anoxic conditions so that the induction of hydrogenase and PS II partially inhibited and electrons mostly come from sources of carbon reserves through plastoquinon. Anaerobic conditions induce expression of hydrogenases [Fe]-in algal cells [45,46] so that continuous hydrogen production can be achieved [14]. Fig. 5 also shows that overall sulfate permease mutants can grow without depleting hydrogen sulfate in the culture medium [47]. Some of the photosystem II inhibitors have also been used to inhibit the activity of water oxidation [48].

Hallenbeck et al. [6] reported that the two photons from the water to form hydrogen gas and simultaneously produce CO_2 reduction by PS I. In the group of green plants, because of the lack of hydrogenase occurred only CO_2 reduction, contrary microalgaes and cyabobacteria have the ability to produce hydrogen, because it has a hydrogenase enzyme. In the process of PS II, the electron is transferred to ferredoxin (Fd) by using solar energy that is absorbed in PS I. Since hydrogenase is very sensitive to oxygen, then the amount of oxygen levels should be controlled below 0.1% so that the production of hydrogen can be stored to maximum yield [6].

2.3. Indirect biophotolysis

Indirect biophotolysis is a biological process that can produce hydrogen from water using a system of microalgae and Cyanobacteria photosynthesis to convert solar energy into chemical energy in the form of hydrogen through several steps: (i) biomass production by photosynthesis, (ii) biomass concentration, (iii) dark aerobic fermentation produces 4 glucose mol hydrogen/mol in the algal cells, together with 2 mol of acetate, and (iv) conversion of 2 mol of acetate into hydrogen. This process can be classified into two distinct groups, one of which is depending on the light and the other is light independent process. The reaction is generally as follows [49]:

$$6H_2O + 6CO_2 + \text{light} \rightarrow C_6H_{12}O_6 + 6O_2 \quad (3)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CH_3COOH + 2CO_2 \quad (4)$$

$$2CH_3COOH + 4H_2O + \text{light} \rightarrow 8H_2 + 4CO_2 \quad (5)$$

The overall reaction as follows:

$$12H_2O + \text{light} \rightarrow 12H_2 + 6O_2 \quad (6)$$

As above shown reactions, we can see that mechanism of photosynthesis process to separate the oxygen and hydrogen undergo through several phases. Oxidation of cyanobacteria stores carbohydrate and produces hydrogen. The energy required to produce hydrogen is also obtained from the starch reserves from previous photosynthetic activity. In stage two, the system sulfur limit C. *reinhardtii* occurs under aerobic conditions separate from the anaerobic conditions, although some of the electrons derived from the starch in the system. This phase was included in the photosynthesis biophotolysis directly due to the operation of cells that are

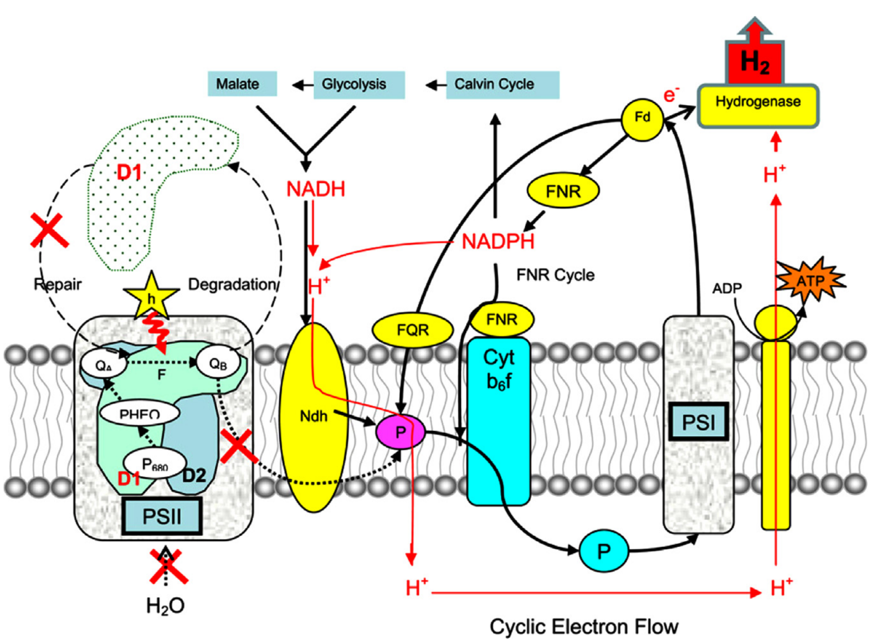


Fig. 5. A schematic of the predicted photobiological pathway of hydrogen production in sulfur deprivation produces anoxic condition for induction of hydrogenase. Adapted from Dasgupta et al. [50].

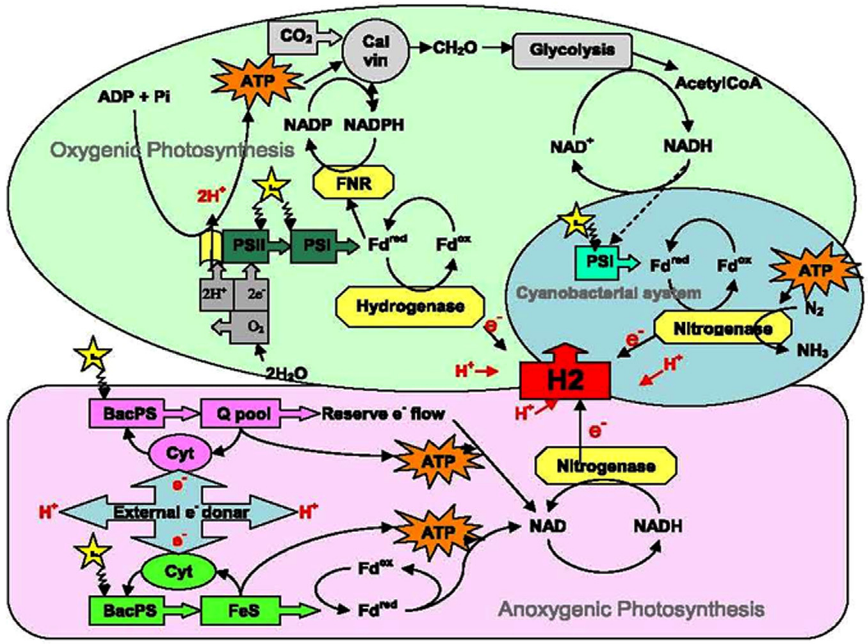


Fig. 6. Schematic processes of electron flow on oxygenic and anoxygenic photosynthesis.
Adapted from Dasgupta et al. [50].

still functioning and provide electrons to hydrogenases during anaerobiosis [42].

Fig. 6 shows the various mechanisms of oxygenic hydrogen production in green algae through hydrogenase and how the blue-green algae hydrogen produced through nitrogenase. Phenomenon of driving electrons is produced from photosynthetic anoxygenic reserve carbon source and hydrogen production in photosynthetic bacteria through nitrogenase, purple bacteria and green bacteria. In Fig. 6, it is also seen that the process of separation phase O_2 and hydrogen evolution in cyanobacteria, carbohydrate is oxidized to produce hydrogen which took place in indirect phophotolysis [50].

In filamentous cyanobacteria, such as the genus Anabaena, spatially separating the two processes by forming heterocysts, nitrogenase is located in heterocycts with functional PS I then catalyzes the formation of hydrogen product. Nitrogenase isoenzymes vary on how many hydrogen ions paired with fixation. Eqs. (7) and (8) showed significant ATP requirement of nitrogenase [51,52].

$$N_2+8H^+ +Fd(red)(8H^-)+16ATP \rightarrow 2NH_3+H_2+Fd(ox)+16ADP+Pi \quad (7)$$

$$8H^+ +8e^- +16ATP \leftrightarrow 4H_2+16ADP+16Pi \quad (8)$$

Electrons donated to PS I in heterocyst derived from carbon transported from neighboring photosynthetic cells, so they do not have their own photosynthetic machinery, which will inhibit the function of the nitrogenase enzyme that catalyzes the O_2-sensitive nitrogen fixation [53].

3. Fundamentals of biological hydrogen production processes by fermentation

There are a variety of biological hydrogen production process, fermentation is one very effective method, because it can be operated and produce hydrogen continuously without the need for light. When compared with hydrogen production through biophotolysis, the hydrogen production by fermentation process has a higher stability and efficiency. In industrial scale, the fermentation process is more appropriate to use because it uses a simple control system, so that the necessary operational costs are minimized. One of the advantages of hydrogen production via fermentation process is using a variety of organic wastes as a substrate, so it can play a dual role of waste reduction and energy production. Thus, hydrogen production through fermentation process has received extensive attention from the researchers and scientists in recent years [54,55].

Biohydrogen production by fermentation processes by using carbohydrates as a substrate has received significant attention from the researchers and scientists in recent years. Here are some reactions of hydrogen production by fermentation of glucose ((9)–(11)) shows that the most desirable end-products is acetate, with production levels of four hydrogen mol^{-1} mol glucose [54,56–59]. Theoretically, the maximum 33% of Chemical Oxygen Demand (COD) can be converted from glucose to hydrogen. The rest of the energy is released as acetate.

$$C_6H_{12}O_6+12H_2O \rightarrow 6HCO_3^- +12H_2+6H^+ \quad \Delta G^0=241\ kj\ mol^{-1} \quad (9)$$

$$C_6H_{12}O_6+4H_2O \rightarrow 2CH_3COO^- +2HCO_3^- +4H_2+4H^+ \quad \Delta G^0=-48\ kj\ mol^{-1} \quad (10)$$

$$C_6H_{12}O_6+2H_2O \rightarrow CH_3CH_2CH_2COO^- +2HCO_3^- +2H_2+3H^+ \quad \Delta G^0=-137\ kj\ mol^{-1} \quad (11)$$

$$C_6H_{12}O_6+3H_2O \rightarrow CH_3CH_2OH+CH_3COO^- +2H_2+3H^+ \quad \Delta G^0=-97\ kj\ mol^{-1} \quad (12)$$

Based on to the theory as shown in reaction (9) above, 12 mol of hydrogen can be produced from one mole of glucose [6,56]. In reactions (9)–(12), the respective Gibbs free energy values at a temperature of 25 °C are highlighted, ΔG° value is calculated based on data from Amend and Shock [60], where production of 12 mol of hydrogen (reaction (9)) is thermodynamically unfavorable. According to Claassen and Van Lier [57], also due to this reaction at hyperthermophilic conditions while the transformation of acetate produced to hydrogen is feasible through photosynthesis

in the partial pressure of hydrogen is very low and operating temperature higher than 40 °C.

In contrast to the former reactions, production of propionate (13) decreases the production of hydrogen [54,58] as was shown experimentally by Shin et al. [61].

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COO^- + 2H_2O + 2H^+ \quad (13)$$

Undesirable consumption of hydrogen (14) or glucose (15) can be caused by the activity of homoacetogens such as *Clostridium aceticum* [62]:

$$2HCO_3^- + 4H_2 + H^+ \rightarrow CH_3COO^- + 4H_2O \quad (14)$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2COO^- + 2H^+ \quad (15)$$

In practice, the fermentation with butyrate as the main product is regarded as the most effective route to produce hydrogen [63,64]. From the experimental results of one type of fermentation that can produce maximum hydrogen is with 2.9 mol of H_2 mol^{-1} glucose by Clostridium species [63,65].

3.1. Photo-fermentation

Photo-fermentation is a fermentative conversion of organic substrates by a diverse group of photosynthetic bacteria that use sun light as energy to convert organic compounds into hydrogen and CO_2. For an example, photo-fermentation with Purple Non-Sulfur (PNS) bacteria can be used to convert fatty acids into hydrogen and small molecules between the products of other gases (namely CO_2). This process takes place in anoxic or anaerobic conditions and by using photosynthetic bacteria and sunlight as energy. Photohydrogen production was performed mainly through four species of PNS bacteria. There are several types of bacteria that can be used in photo-fermentation process such as bacteria *Rhodobacter sphaeroides* [66], *Rhodopseudomonas palustris* [67], *Rhodobacter capsulatus* [68], and *Rhodospirillum rubrum* [69]. By using small molecule organic acids like acetate, lactate and butyrate as carbon and energy source of light that can change the carbon source to produce hydrogen [70,71]. While dark fermentation is the conversion of organic substrates by various groups of bacteria that take place in the dark (without the presence of light) with a series of biochemical reactions and takes place under anaerobic conditions [72].

In the photo-fermentation process, PNS bacteria is a group of photosynthetic bacteria has some advantage over compared to cyanobacteria and algae. These bacteria use enzyme nitrogenase to catalyze nitrogen fixation for reduction of molecular nitrogen to ammonia. Nitrogenase has interesting property that it can evolve hydrogen simultaneously with nitrogen reduction. Stressful concentrations of nitrogen are therefore required for hydrogen evolution. Photo-heterotrophs make use of energy from sunlight to oxidize organic compounds and generate the electron potential needed to drive hydrogen production. By utilizing energy from the sun to drive thermodynamically unfavorable reactions, PNS bacteria can potentially divert 100% of electrons from an organic substrate to hydrogen production. In this processes, photo-heterotrophs typically utilize the smaller organic acids that are often produced but not metabolized, during dark fermentation. Thus, waste streams from photo-fermentation contain fewer by products as the organic compounds are fully reduced to form H_2 and CO_2 [73].

In principle, photofermentations able to fully convert organic compounds into hydrogen, even against a relatively high hydrogen partial pressure, because hydrogen evolution is driven by ATP-dependent nitrogenase and ATP formed is capture light energy through photosynthesis. Some researchers have conducted a study that non-sulfur purple photosynthetic bacteria capture light energy and use it to convert organic acids into hydrogen quantitatively [72,74].

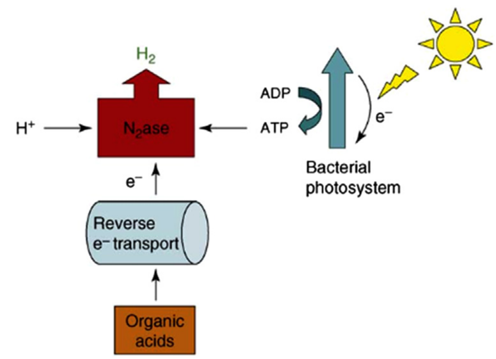


Fig. 7. Photo-fermentation processes by photosynthetic bacteria.
Adapted from Hallenbeck et al. [42].

In Fig. 7, we can see that the non-sulfur photosynthetic bacteria carry out a photosynthetic anaerobic purple, then captured using solar energy to generate ATP and high energy electrons through electron flow through, which then reduces ferredoxin. Reduction of ATP and reduced ferredoxin drive the hydrogen protons with nitrogenase. The organism is unable to obtain electrons from water and therefore the use of organic compounds, usually organic acids, as substrates [42].

Some researchers reported that although the stoichiometric conversion of several organic acids into hydrogen on photofermentation process can be obtained, but the light conversion efficiency and the level of production volume is still low. Results of recent studies have shown that to produce the maximum hydrogen, it is suggested to use two-stage system of photo-dark fermentation [75–77]. Moreover, photo-fermentation bacteria can utilize short chain organic acids which are produced in dark-fermentation, a combination of dark- and photo-fermentation can be achieved the highest theoretical hydrogen yield of 12 mol H_2/mol hexose, although results are still far below the stoichiometric [78].

One group of proteobacteria which have photosynthetic pigments and capable of photosynthetic are categorized as Purple Sulfur Bacteria (PSB). They are anaerobic or microaerophilic, and are often found in hot springs or stagnant water. Unlike plants, algae and cyanobacteria, they do not use water as their reducing agent, and consequently, do not produce oxygen. Instead, they use hydrogen sulphide or other reduced sulphur compounds as electron donor, which is oxidized to produce granules of elemental sulphur, which become visible in cells [79].

Current research on biohydrogen production using anaerobic photo-fermentation can be seen in Table 2. The production of hydrogen from acetate using photo-fermentation is a batch reactor type that has been studied at various laboratories [86,92,93]. Hydrogen production by Rhodopseudomonas palustris WP 3-5 in a serial photo bioreactor fed with hydrogen fermentation effluent has been studied by Lee et al. [87]. The production of hydrogen yield in cylindrical reactor production by *Rhodobacter capsulatus* with pigment content manipulation was then introduced by Ma et al. [89]. The semi-continuous photofermentative H_2 production by *Rhodobacter sphaeroides* from succinate was more focused by Kim et al. [90] and the bifocculation of photo-fermentative bacteria induced by calcium ion for enhancing hydrogen production using continuous bioreactor has been done by Xie et al. [92]. Based on Table 2, many works have been conducted to improve the hydrogen yields and behavior of bacteria, such as Mixed photosynthetic culture [81], *Rhodopseudomonas faecalis* RLD-53 [82,83,88,92], *Rhodobacter sphaeroides* O.U.001 [71,86], *Rhodopseudomonas palustris* CQK 01 [84], *Rhodopseudomonas palustris* WP 3-5 [87], *Rhodobacter capsulatus* with cbb3 gene [85,89] and *Rhodobacter sphaeroides* KD131 [90,91].

Table 2
Comparison of the maximum H_2 yield obtained in various types of H_2-producing reactor on anaerobic photo-fermentation.

Reactors	Bacterial strains	Substrate	Operating conditions		Maximum H_2 yield	Ref.
			pH	Temp. (°C)		
Batch	*Rhodobacter sphaeroides*	Sodium lactate	8.9	30	2.4 mg/l	[80]
Batch	Mixed photosynthetic culture	Acetate and butyrate	6–7	34	3.51 mol/Kg CODR-day	[81]
Batch	*Rhodopseudomonas faecalis* RLD-53	Acetate	7	35	2.61 mol H_2/mol acetate	[82]
Photobioreactor	*Rhodobacter sphaeroides* O.U. 001	Malate, acetate and butyrate	6.7	30–33	24 ml H_2/l h	[71]
Batch	*Rhodopseudomonas faecalis* strain RLD-53	Acetate	7	35	3.17 mol H_2/mol acetate	[83]
Biofilm-based photobioreactor	*Rhodopseudomonas palustris* CQK 01	Glucose	7	25	0.2 mol H_2/mol glucose	[84]
Tubular photo bioreactor-fed batch	*Rhodobacter capsulatus*	Acetate	≤ 8	10–35	0.6 mol H_2 per mole of acetic acid fed	[85]
Batch	*Rhodobacter sphaeroides* O.U. 001	Brewery wastewaters	7–7.2	28 ± 2	2.24 l H_2/l medium	[86]
Continuous	*Rhodopseudomonas palustris* WP 3-5	Synthetic wastewater	6.8	28 ± 35	205 mL H_2 L/d	[87]
Sequencing batch reactor	*Rhodopseudomonas faecalis* RLD-53	Acetate	7	35 ± 1	3.12 mol H_2/molacetate	[88]
Cylindrical	*Rhodobacter capsulatus* with cbb3 gene	Acetic and butyric acid	6.8	35	3752.7 mL H_2 L/L	[89]
Semi-continuous	*Rhodobacter sphaeroides* KD131	Succinate	7.5 ± 0.2	30	3.7 mol H_2/mol succinate	[90]
Continuous	*Rhodobacter sphaeroides* KD131	Succinate	7.5 ± 0.2	30	2.3 mol H_2/mol succinate	[91]
Batch and continuous	*Rhodopseudomonas faecalis* RLD-53	Acetate	7	35 ± 1	2.64 mol H_2/mol acetate	[92]
Batch	*Rhodobacter sphaeroides* KD131	Hexose	7	30	8.35 mol H_2/mol hexose	[93]

3.2. Dark-fermentation

Dark fermentation is the fermentative conversion of organic substrate and biomass materials to produce biohydrogen which takes place in anaerobic conditions and without the presence of light. It is complex process manifested by various groups of bacteria by involving a series of biochemical reactions [94]. Dark hydrogen fermentation has several advantages compared with other biological methods of hydrogen production such as photosynthetic and photo fermentation because of its ability to produce hydrogen continuously without the presence of light, higher hydrogen production rate, process simplicity, lower net energy input and utilization of low-value waste as raw materials [95–98].

Dark fermentation produces hydrogen from organic compounds by anaerobic microorganisms [61,99–102]. Dark fermentation can also produce hydrogen from organic waste as shown in the following equation [56,103]:

$$C_{12}H_{22}O_{11} + 9H_2O \rightarrow 4CH_3COO^- + 8H^+ + 4HCO_3^- + 8H_2 \quad (16)$$

In order to increase yield more hydrogen in the dark fermentation process, it is necessary to control several parameters namely pH, organic food, nutrition feed rate, temperature, Solids Retention Time (SRT), and P_{H_2}. One of the most important parameters on hydrogen production is pH, because pH is one factor influence on the activities of the enzyme hydrogenase. There have been several studies reported that the hydrogenase activity are directly correlated with dark fermentation of hydrogen, this indicates that the pH plays a very important role on hydrogen production [104]. Many papers have reported that the effect of pH in fermentative hydrogen production from glucose and sucrose using mixed microflora [105–109].

Many studied have been reported that the pH value is maintained in conditions of low and shortening the shorter SRT, thus limiting the growth of methanogens. In general, studies based on several studies dark fermentation, pH value was maintained at a pH range of 5.5 to 8.0 either by adjusting the initial pH, buffer usage, or using an automatic pH controller. By applying these techniques, the maximum conversion efficiency has been increased by 60–70% [110]. Fang and Liu [111] also have obtained the optimum pH value in the range of 5.5 to the production of hydrogen in chemostat culture using a mixture of holding time for 6 h, so that the growth of methanogens can be slowed.

Several studies have been conducted for the hydrogen production on a batch, anaerobic sequencing batch reactor (AnSBR), fed-batch, fluidized bed bioreactor (FBR), continuously stirred tank reactor (CSTR) and continuous dark fermentation with different types of raw materials. Sagnak et al. [112] fermented acid hydrolyzed waste ground wheat using anaerobic sludge as bacterial strains for hydrogen gas production by anaerobic dark fermentation. Microbial tri-saccharides species and anaerobic digester sludge were used for dark fermentation of hexose in batch systems has been done by Que'me'neur et al. [141]. Ozmihci et al. [142] used Clostridium butyricum-NRRL 1024 from waste wheat starch for dark fermentative bio-hydrogen in a continuous production. Table 3 also summarizes the comparison of the maximum hydrogen yield obtained in various types of hydrogen producing reactor on anaerobic dark fermentation. Anaerobic sequencing batch reactor (AnSBR) experiments to produce hydrogen from food waste [124], liquid swine manure [121], cassava starch [134], glucose and xylose [152]. A number of studies are reported in the literature for the production of hydrogen using batch experiments from xylose [143], cheese whey powder [144], grass silage [145], dry grass [146], food waste [147], distillery wastewater [148] and beef extract [150]. There are also works with CSTR setups to produce hydrogen from sweet sorghum extract [138], xylose [143], cellulose [151] and glycerol [153] as shown in Table 3.

3.3. Photo-dark fermentation

The main problem faced by using a dark fermentation biohydrogen production is low yield and energy efficiency, for example in dark fermentation for 1 mol hexose can only produce 2 to 4 mol of hydrogen with acetate and butyrate as byproduct [154]. In addition to producing hydrogen also byproducts contain many organic acids, which lead to energy waste and environmental pollution. While in photofermentation, organic acids can be used side by photosynthetic bacteria for further processing and then converted into hydrogen production [155]. Various efforts have been done so that new approaches such as byproduct of organic acid produced by fermentation dark for further methane and hydrogen production in other processes [156–158].

The best solution to solve this problem is by using sequentially between dark fermentation process and photofermentation. This concept is very promising for the production of biohydrogen

Table 3
Comparison of the maximum biohydrogen yield obtained in various types of H_2-producing reactor on anaerobic dark-fermentation.

Reactors	Bacterial strains	Substrate	HRT (h)	pH	Temp (°C)	Maximum H_2 yield	Refs.
CSTR	*Clostridium butyricum* GS2	Starch	12	6.5	37	0.52 L/h/L and 13.2 mmol H_2/g total sugar	[113]
Batch	Municipal wastes, methanogene bacteria	Glycerol	–	6	37	0.41 mol H_2/mol glycerol	[114]
FBR	Sewage sludge	Sucrose	6	–	–	4.26 mol H_2/mol sucrose	[115]
Batch	POME sludge	Food waste	–	7	55	593 mL H_2/g carbohydrate	[116]
Fed-batch	Clostridium sp.	Swine manure	16	5	35	18.7×10^{-3} g H_2 per g TVS	[117]
Batch	Compost	Sucrose	–	–	22	4.3 mol H_2/mol sucrose	[118]
Batch	*Escherichia coli*, DJT135	Fructose, sorbitol, glucose	–	6.5	35	1.27, 1.46 and 1.51 mol H_2/substrate	[119]
Fed-batch	Ground wheat	Starch, glucose				465 mL H_2/g starch, 3.1 mol H_2/mol glucose	[120]
Batch	Cow dung	Glucose	8.34	5.0	33.5	2.15 mol H_2/mol glucose	[121]
Continuous	Microbial consortium	Glucose	4	5.5	110	2.30 mol H_2/mol glucose	[122]
IBRCS-CSTR	Inocula – digested sludge	Glucose	8	5.5–6.5	37	2.8 mol H_2/mol glucose	[123]
AnSBR	Seed sludge	Food waste	36	5.3 ± 1	35 ± 1	0.5 mol H_2/mol hexoseadded	[124]
Batch	Clostridium sp. R1	Carbohydrate	–	6	30	3.5 mol H_2/mol cellobiose	[125]
ACSTR	*Clostridium butyricum*	Glucose	5–10	4.9	37	1.3 mol H_2/mol glucose	[126]
Batch	Clostridium sp. DMHC-1	Sludge of distillery waste	–	5.0	37	3.35 mol H_2/mol glucose	[127]
Batch	*Clostridium butyricum* and *Clostridium bifermentans*	Riverbed sediments	–	6	37	2.3 mol H_2/mol glucose	[128]
Batch	*Bacillus coagulans* IIT-BT S1	Sludge as substrate	12	6	37	37.16 mlH_2/g COD consumed	[129]
Batch	River sludge	Apple pomace	–	4.5	5.0	134.04 ml/g total solid (TS)	[130]
CSTR	Inoculated sludge	Purified terephthalic acid	6	6.0	35 ± 1	0.073 L/g MLVSS d	[131]
AnSBR	Seed sludge from a dairy manure	Liquid swine manure	16	5.0	37 ± 1	1.50 mol H_2/mol glucose	[132]
Batch	Rice rhizosphere microflora	Apple pomace	–	6.0	35	2.3 mol H_2/mol hexose	[133]
AnSBR	Seed sludge from cassava wastewater	Cassava starch		5.5	37	186 ml H_2/g COD	[134]
Batch	Diverse microflora	Activated sludge			1. 37 2. 55	(1). 2.18 mol H_2/mol glucose (2). 1.25 mol H_2/mol glucose	[135]
Batch	River sludge	Apple pomace	–	7.0	37	101.08 ml/g total solid (TS)	[136]
Batch and sequenced-batch	*Clostridium butyricum* CWBI1009	4. Glucose 5. Starch		(1). 5.2 (2). 5.6	30	1. 1.7 mol H_2/mol glucose 2. 2.0 mol H_2/mol hexose	[137]
CSTR	Sorghum bicolor L. Moench	Sweet sorghum extract	12	4.7	35	0.93 ± 0.03 mol H_2/mol glucose	[138]
Batch	Anaerobic sludge	Waste ground wheat	–	7	37	1.46 mol H_2/mol glucose	[139]
Batch	Anaerobic sludge	Acid hydrolyzed wheat starch and sugar	–	5.5	30	200 ml H_2/g sugar	[140]
Batch	Anaerobically-digested sludge	Tri-saccharides		5.5	37	1.84 mol-H_2/mol-hexose	[141]
Continuous	*Clostridium butyricum*-NRRL 1024 and *Clostridium pasteurianum*-NRRL B-598	Ground wheat starch	6–60	5.5	30	109 ml H_2 gT/S	[142]
1. Batch 2. CSTR	*Clostridium acetobutylicum* and *Citrobacter freundii*	Xylose	–	6.8	45	1. 0.71 mol H_2/mol xylose 2. 1.97 mol H_2/mol xylose	[143]
Batch	Anaerobic sludge	Cheese whey powder	–	5.5	55	1.03 mol H_2/mol glucose	[144]
Batch	Bacterial hydrolysis	Grass silage	–	7	37	37.8 ± 5.8 mLH_2/g silage	[145]
Batch	*Clostridium pasteurianum*	Dry Grass	–	7	35	72.21 mLH_2/g-dry grass	[146]
Batch	Sewage sludge	Food waste		6.0 ± 1	35 ± 1	2.26 mol-H_2/mol-hexose	[147]
Batch	Anaerobic digested sludge	Distillery wastewater		5.5	37	1 L H_2/L medium	[148]
Semi-continuous	Seed anaerobic sludge	Glucose		6–8	35	7 mmol H_2/gdwt-h	[149]
Batch	Bacillus sp. and Brevundimonas sp.	Beef extract		5.0–6.8	35	1.94 mol H_2/mol glucose	[150]
CSTR	Anaerobic mixed micrflora	Cellulose	10	5.86 ± 0.1	55 ± 1	12.28 mmol H_2/g cellulose	[151]
AnSBR	Anaerobic sludge blanket reactor	1. Glucose 2. Xylose	1.7	5.5		1. 2.89 ± 0.18 mol H_2/mol glucose 2. 1.94 ± 0.17 mol H_2/mol xylose	[152]
CSTR	*Clostridium pasteurianum*	Glycerol	–	7	35	0.77 ± 0.05 mol H_2/mol glycerol	[153]

because hydrogen production is greater than the dark phase of the fermentation process or a single photofermentation. So, the two-stage process combining dark and photofermentation can improve the hydrogen production, theoretical from 4 to 12 mol H_2/mol hexoses and from 2 to 10 mol of H_2/mol pentose [159]. During the dark fermentation of carbohydrate containing substrate is converted into organic acids, CO_2 and hydrogen by mesophilic and thermophilic bacteria. In the second stage, dark fermentation waste containing organic acids such as acetic and lactic bacteria used in photofermentation by photosynthetic or Purple Non-Sulfur (PNS) for hydrogen production further. Su et al. [160] also reported that sequential technological dark and photo-fermentative been used to increase the yield of hydrogen from glucose and starch cassava.

There are studies concentrate on the comparison of hydrogen yield obtained from sequential dark-photo fermentation systems reported in literatures. Several studies have been conducted for the hydrogen production on a batch reactor, continuous, CSTR and fed-batch reactor using different raw materials. Experimental work and study have been conducted and successfully produced hydrogen gas on a two-stages sequential in batch reactor dark-photo fermentation process using glucose [160,166] and sucrose [162]. A number of studies are also reported in the literature for the production of hydrogen using batch experiments from cassava starch [165,168], corncob [155], molasses [169], rice straw [170] zeolite [173], chlorella pyrenoidosa raw biomass [174] and water hyacinth [175]. Sagnak et al. [171] used Rhodobacter sphaeroides (NRRL-B 1727) and anaerobic sludge from ground waste wheat for

dark-photo fermentative bio-hydrogen production. Experiments with CSTR to produce hydrogen from sucrose [164] and fed-batch reactor systems from wheat starch has also been carried out by some researcher [167,172], as tabled out in Table 4.

4. Hybrid biological hydrogen production by electrochemical processes

Electrochemical methods offer some advantages over traditional chemical treatment: less coagulant ion is required, less sludge is formed, and electrocoagulation equipment is very compact; thus, suitable for installation where the available space is rather limited. Furthermore, the convenience of dosing control only by adjusting current makes automation quite easy [176–178].

Electrocoagulation is an electrochemical method of treating polluted water whereby sacrificial anodes dissolve to produce active coagulant precursors (usually aluminum or iron cations) into solution. Additionally, electrolytic reactions evolve gas (usually as hydrogen bubbles) at the cathode that can enhance the process; this effect is known as electroflotation [179–181].

One possible reason is the energy demand of the electrocoagulation process. Hydrogen is a main byproduct of the electrocoagulation process as it is generated at the cathodes by water electrolysis. With an effective gas–liquid–solid separation process, high quality hydrogen can be recovered from the electrocoagulation process and used as an energy source or as a reactant for industrial processes. Production of hydrogen by the electrochemical process is not limited to carbohydrates, such as in the fermentation process, because any biodegradable dissolved organic matter can theoretically be used in this process to produce hydrogen from the complete oxidation of organic material. Electrocoagulation is one way to produce hydrogen as well as an alternative treatment method for wastewater. Occurrence of electrocoagulation method is to separate water into hydrogen and oxygen elements by passing an electric current between two electrodes in water [182,183].

$$2H_2O \rightarrow O_2 + H_2 \quad (17)$$

Electrocoagulation is a complex process occurring via electrolytic reactions at electrode surfaces and formation of coagulants in the aqueous phase [184]. Electrocoagulation process is based on the formation of thickeners (hydroxyl metals) in wastewater by dissolving the anode as shown in Fig. 8 [185].

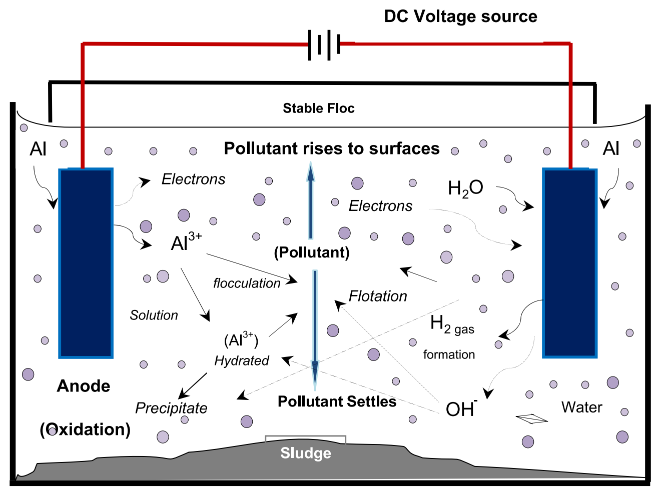


Fig. 8. Interaction inside cell electro-coagulation [185].

Table 4
Comparison of biohydrogen yield obtained from sequential dark-photo fermentation systems reported in the literature.

Operation mode	Microorganism used in dark fermentation	Microorganism used in photo fermentation	Carbon source	pH	Temp. (°C)	Total maximum H_2 yield	Refs.
PhBR1	*Rhodobacter capsulatus* B10	*Rhodobacter capsulatus* B10	Potato homogenate	5.0	28	5.6 mol mol/1 glucose	[75]
Batch	Microflora	*Rhodobacter sphaeroides* SH2C	Sucrose	6 and 7	38 and 30	6.63 mol H_2/mol sucrose	[161]
Batch	Anaerobic sludge	*Rhodobacter sphaeroides*-RV	Ground wheat starch	6–7	30	156.8 ml H_2 g^{-1} starch	[154]
Batch	*Clostridium butyricum*	*Rhodopseudomonas palustris*	Glucose	7 ± 0.02	35	5.48 mol H_2/mol glucose	[160]
Batch	*Clostridium butyricum* CGS5	*Rhodopseudomonas palustris* WP3–5	Sucrose	7.5	37	5.45 mol H_2/mol hexose	[162]
CSTR	*Clostridium butyricum* CGS5	*Rhodopseudomonas palustris* WP3-5	Sucrose	7.1	32	5.81 mol H_2/mol hexose	[163]
CSTR	*Clostridium butyricum* CGS5	*Rhodopseudomonas palutris* WP3-5	Sucrose	6.5	37	11.61 mol H_2/mol sucrose	[164]
Batch	Clostridium species	*Rhodopseudomonas palustris* species	Cassava starch	5.5–7.5	25–45	840 ml H_2/g starch (6.07 mol H_2/mol hexose)	[165]
Batch	Dairy manure	*R. sphaeroides*	Corncob	7	35–36	713.6 ± 44.1 mL H_2/g-COD	[155]
Batch	*Clostridium butyricum*	*Rhodopseudomonas faecalis* RLD-53	Glucose	7	35	122.4 ml H_2/vessel	[166]
Fed-batch operation	Anaerobic sludge (AN)	*Rhodobacter sphaeroides*-NRRL	Wheat starch	7.5	35	201 ml H_2 g/1 starch	[167]
Batch	*Clostridium butyricum*	*Rhodopseudomonas palustris*	Cassava starch	7 ± 0.02	30 ± 0.5	2.91 to 6.07 mol H_2/mol hexose	[168]
Batch	*Rhodobacter capsulatus* hup⁻ YO3	*Rhodobacter capsulatus* DSM 1710	Molasses	6.4	35	0.50 mmol H_2/L_c h	[169]
Batch	Clostridium butyricum	*Rhodopseudomonas palustris*	Rice straw	6.5 ± 0.1	35	463 ml/g TVS	[170]
Continuous	Anaerobic sludge	*Rhodobacter sphaeroides* (NRRL-B 1727)	Ground waste wheat	7	30 ± 1	3.4 mol H_2/mol glucose	[171]
Fed-batch operation	*Rhodobacter capsulatus* YO3	*Caldicellulosiruptor saccharolyticus*	Sugar beet thick juice	6.5	≤ 40	1.12 mmol H_2/L_c h	[172]
Batch	*Clostridium butyricum*	*Arthrospira platensis*	Zeolite	6.5 ± 0.05	35	96.6 to 337.0 ml H_2/g DW	[173]
Batch	Bacteria (HPB), PSB, and MPB	Bacteria (HPB), PSB, and MPB	*Chlorella pyrenoidosa* raw biomass	8.0 ± 0.1	35 ± 1.0	198.3 ml/g TVS	[174]
Batch	Bacteria (HPB), PSB, and MPB	*Rhodopseudomonas palustris*	Water hyacinth	6.0 ± 0.1	35 ± 1.0	112.3 ml/g TVS to 751.5 ml/g TVS	[175]

Reaction that occurs in electrocoagulation using aluminum electrodes are as follows [186]:

For aluminum electrodes: Al + 3e → Al^{3+}

$$\text{Anode (in alkaline)}: Al^{3+} + 3(OH)^- \rightarrow Al(OH)_3 \quad (18)$$

$$\text{Anode (in acid)}: Al^{3+} + 3H_2O \rightarrow Al(OH)_{3(s)} + 3H^+ \quad (19)$$

For iron electrodes: (Fe + 2e → Fe^{2+}):

$$\text{Anode (in alkaline)}: Fe^{2+} + 2(OH)^- \rightarrow Fe(OH)_2 \quad (20)$$

$$\text{Anode (in acid)}: 4Fe^{2+} + O_2 + 2H_2O \rightarrow 4Fe^{3+} + 4OH^- \quad (21)$$

Subsequent reaction of oxygen into:

$$2H_2O + 4e \rightarrow O_2 + 4H^+ \quad (22)$$

At cathode, subsequent reaction of oxygen into:

$$2H_2O + 2e \rightarrow H_2 + 2OH^- \quad (23)$$

Electrolysis using aluminum as the anode and cathode will result in thickening of $Al(OH) \cdot xH_2O$ and at the top of the hydrogen gas will be produced that will take the water of dissolved solids in the liquid waste [187,188]. Other than hydrogen gas is expected to be taken and used for other needs [189].

Anodic process resulted in aluminum metal dissolved and formed molecular ion Al^{3+}. Ions Al^{3+} formed in solution will produce a solid water-insoluble $Al(OH)_3 \cdot xH_2O$ through hydrolysis reactions [187,190].

$Al(OH)_3 \cdot xH_2O$ formed in solution can serve as thickeners for coagulation–flocculation processes that occur in the next process in the electrolysis cell. After coagulation–flocculation process is completed, the contaminated materials within the waste water will settle by itself [189,190].

Electrocoagulation process has several advantages such as not requiring a large region for the treatment of waste water, requires only simple equipment and easy to obtain, process optimization is easy, unnecessary chemical additives and produce oxygen gas and hydrogen can help in the treatment of the flotation process [191], as well as help in the process of waste water treatment, electrocoagulation also has the advantage of hydrogen gas that is produced can be used as an energy source. Hydrogen gas in the process electrocoagulation rarely taken and often researchers use electrocoagulation only for wastewater treatment and let the hydrogen escape to the environment.

Production of hydrogen from protons and electrons are produced directly by bacteria with increasing electrochemical potential in the cathode Microbial Fuel Cell (MFC). The most interesting part of the process of electrochemical is the occurrence of two simultaneous processes that produce hydrogen gas and electrocoagulation process.

Hydrogen can be produced from certain forms of biomass by biological fermentation [192], but yields are low. The maximum hydrogen production from fermentation, assuming only acetate or butyrate is produced from glucose, is

$$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CO_2 + 2C_2H_4O_2 \quad (24)$$

$$C_6H_{12}O_6 \rightarrow 2H_2 + 2CO_2 + 2C_4H_8O_2 \quad (25)$$

4 mol of H_2/mol glucose could be obtained if only acetate was produced, but only 2 mol H_2/mol if butyrate is the sole end product. Current fermentation techniques produce a maximum of 2–3 mol H_2/mol glucose. Thus, most of the remaining organic matter is essentially wasted as a mixture of primarily acetic and butyric acids, despite a stoichiometric potential of 12 mol H_2/mol glucose [193]. The largest hydrogen yield theoretically possible using microorganisms (without an external source of energy) is therefore at 4 mol H_2/mol glucose based on production of acetic acid. Higher yields can be achieved using photobiological process and supplemental light, or using pure enzymes, but neither of these methods so far show promise for economical production of hydrogen [194–196]. Moreover, of all the different types of biomass available for making hydrogen, only materials rich in carbohydrates such as glucose are suitable fermentation substrates.

Bio-electrochemical system is an alternative technology using microorganisms as electrochemical catalyst. Microorganisms are capable of catalyzing the oxidation-reduction reaction at the anode and cathode electrodes. Bio-electrochemical systems (BESs) are divided into two major groups which are Microbial Fuel Cells (MFCs) and Microbial Electrolysis Cells (MECs).

4.1. Microbial fuel cell

MFC and MEC are among such bioelectrochemical systems. Together, MFC and MEC could be represented by the acronym MxC. Performance of MxC largely depends on anaerobic biofilm occupied by anodophilic (electrogenic) microorganisms, which transfer electrons to the anode during their metabolism [197]. Though anodic compartments in all MxC are similar, the cathode reactions differ. MFC operate with cathodes exposed to air, resulting in oxygen reduction reaction at the cathode and electricity production [198]. In contrast, MEC require a small additional input of electrical energy provided by an external power supply to facilitate the reaction of hydrogen formation on the cathode [199]. A MFC consists of two electrodes (anode and cathode), where bacteria grows on organic materials dissolved in the anode chamber in anaerobic conditions. Due to activities of the bacteria, chemical energy from organic matter in the wastewater is converted into electrical energy. Microorganisms oxidize substrates to produce electrons and then transfer to the anode electrode. As the result, electrons flow through an external circuit to the cathode electrode and produce a measurable electrical current [200].

By electrochemically augmenting the cathode potential in a MFC circuit [201] it is possible to directly produce hydrogen from protons and electrons produced by the bacteria. This approach greatly reduces the energy needed to make hydrogen directly from organic matter compared to that required for hydrogen production from water via electrolysis. In a typical MFC, the open circuit potential of the anode is ~ -300 mV [200,202]. If hydrogen is produced at the cathode, the half reactions occurring at the anode and cathode, with acetate oxidized at the anode, are as follows:

$$\text{Anode}: \; C_2H_4O_2 + 2H_2O \rightarrow 2CO_2 + 8e^- + 8H^+ \quad (26)$$

$$\text{Cathode}: \; 8H^+ + 8e^- \rightarrow 4H_2 \quad (27)$$

Producing hydrogen at the cathode requires a potential of at least $E^0 = -410$ mV at pH 7.0 [203], so hydrogen can theoretically be produced at the cathode by applying a circuit voltage greater than 110 mV (i.e., 410–300 mV). This voltage is substantially lower than that needed for hydrogen derived from the electrolysis of water, which is theoretically 1210 mV at neutral pH. In practice, 1800–2000 mV is needed for water hydrolysis (under alkaline solution conditions) due to over potential at the electrodes [204].

It is shown here that this biochemical barrier can be circumvented by generating hydrogen gas from acetate using a completely anaerobic microbial fuel cell (MFC). More than 90% of the protons and electrons produced by the bacteria from the oxidation of acetate were recovered as hydrogen gas, with an overall Coulombic efficiency (total recovery of electrons from acetate) of 60–78%. This is equivalent to an overall yield of 2.9 mol H_2/mol acetate (assuming 78% Coulombic efficiency and 92% recovery of electrons as hydrogen). This bio-electrochemically assisted microbial system, if combined with hydrogen fermentation that produces 2 to 3 mol of H_2/mol

glucose, has the potential to produce 8 to 9 mol H_2/mol glucose at an energy cost equivalent to 1.2 mol H_2/mol glucose [205].

4.2. Microbial electrolysis cells

A MEC is a slightly modified MFC, where a small amount of electricity is applied to the anode chamber to suppress the production of methane and oxygen is kept out of the cathode chamber to assist bacterial oxidation of organic matter present in wastewater to produce hydrogen. While MEC has tremendous potential, the development of this technique is still in its infancy. Information about the anode materials and microorganisms used in MFCs are also applicable to MEC systems due to their similar anodic process. Yet, efficient and scalable designs are required and investigated by biologists for the successful applications of the microbial electrolysis process [206].

5. The unique and advantages of biohydrogen production processes

Table 5 highlights characteristics, uniqueness and common criterias for each of the biohydrogen production processes. Every biohydrogen production processes have its own advantages and disadvantages. The best approach to maximize the production of hydrogen gas is to combine these three processes. The proposed process shall be executed in sequence, namely the photo-fermentation, dark-fermentation and bio-electrochemical processes. This concept is regarded as a very promising approach for the maximum biohydrogen production when compared to the dark-fermentation or photo-fermentation phase alone. By using each process individually, the maximum hydrogen gas obtained is only at the range of 60–70%. But, by combining these three processes in sequence, it can increase the hydrogen gas production up to 91% [207].

6. Conclusion and perspectives

In conclusion, biological hydrogen production is the most challenging undertaking issue in the last decade, while world energy demand increases, fossil fuel resources is reduced and the need to minimize greenhouse gas is becoming increasingly concerned. Hydrogen gas will be one of the realistic energy in future as the growing science of biotechnology. It can overcome environmental concerns and social changes. It is a fact that hydrogen is a clean and efficient energy carrier, produces zero emission, and can be generated by managing the renewable sources such as biomass and waste.

Since steam reforming or partial oxidation hydrocarbon fossil fuels operate at high temperatures the chemical methods require very high operating costs. It is necessary to develop a new process to obtain hydrogen fuel with a low production cost. Biological method has potential as an alternative to the current renewable technologies because it offers promising advantages such as operating under mild conditions and with a specific acceptable conversion rate. The sources of raw materials can be obtained from a variety of organic-based starch, cellulose containing solid wastes, food industrial wastewater, industrial waste biodiesel, Palm Oil Mill Effluent (POME), etc.

There are various technologies used for biological hydrogen production to include the biophotolysis of water by cyanobacteria and green micro algae, photo fermentation, dark fermentation, photo-dark fermentation and bio-electrochemical processes. Hydrogen production using biophotolysis systems by cyanobacteria and green micro-algae become an alternative method of gaseous energy recovery and have the potential to be applied in the production of renewable energy. Research on photobiological hydrogen metabolism has increased significantly; further studies need to be more innovative to increase the effectiveness of photobioreactors. Direct biophotolysis is a biological process that

Table 5
Comparison of the unique or common processes of biohydrogen production by cyanobacteria and green micro algae, photo-fermentation, dark-fermentation and bio-electrochemical process.

No	Processes	Common	Unique	Refs.
1	Cyanobacteria and green micro algae	1. Uses carbohydrates to store energy 2. Take place in anaerobic condition	1. Using biophotolysis process 2. No requirement of adding substrate as nutrients 3. Only using water, CO_2 and sunlight energy as a source of energy 4. H_2 can be produced directly from water and sunlight 5. It has the ability to fix N_2 from atmosphere	[12–15]
2	Photo-fermentation	1. Uses organic wastes as a substrate 2. This process takes place in anoxic and anaerobic conditions	1. Can use a variety of organic wastes as a substrate 2. Using photosynthesis bacteria 3. Using sunlight as energy to convert organic compounds into hydrogen 4. A wide spectral energy can be used by photosynthetic bacteria	[42,72–74,79]
3	Dark-fermentation	1. Takes place in anaerobic conditions 2. Using organic substrate and biomass to produce biohydrogen	1. It is ability to produce hydrogen continuously without the presence of light 2. This process take place in dark condition 3. Higher hydrogen production rate 4. Process very simplicity 5. Lower energy input 6. Can use low-value organic waste as raw material 7. No oxygen limitation 8. Can produce several metabolites as by-products	[94–98,104,154]
4	Bio-electrochemical process	1. Takes place in anaerobic conditions 2. Using organic substrate to produce biohydrogen	1. This process is also used to remove organic contaminants in wastewater 2. It is possible to directly produce hydrogen from protons and electrons produced by the bacteria 3. More than 90% of protons and electrons produced by the bacteria	[199,206,207]

can produce hydrogen directly from water, even though productivity of hydrogen production is relatively limited, but has provided new knowledge about the phenomenon hydrogenases enzymes, biomaterial and the nature of electron carriers in the photosynthesis system. On the other hand, indirect biophotolysis has its advantages and potential to enable hydrogen energy cogeneration involves steps of photosynthesis and biomass production of dark anaerobic fermentation of biomass to produce hydrogen.

In the dark fermentation, the conversion of organic compounds to hydrogen gas through a complex process involves a diverse group of bacteria with complex series of biochemical reactions. While the photo-fermentation, the conversion of organic compounds into hydrogen gas can only take place in the presence of light. By combining the two processes, it is currently the most interesting approach that can be used to increase the production of hydrogen gas. In this process, besides having higher levels of hydrogen production, fast and simple operation, it can be used with a variety of organic wastes as substrates. Thus, compared with the production of hydrogen through the process of photosynthesis, the production of hydrogen via fermentation is more suitable to be used to produce cleaner energy and to treat organic waste more efficiently.

One of the advantages of the MEC, it is able to produce high hydrogen production, gas capture efficiency is up to 91%. Performance of the MEC is determined by the physiology of microorganisms and on the other hand is also determined by the physical chemical transport processes involved. It comes with results of high H_2 and able to provide multiple benefits in terms of maximum H_2 yield and minimize the BOD of the waste treated. It is one of the major advantages when compared with the fermentation process.

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